ORIGINAL RESEARCH

Combining Thyroglobulin Levels in Lymph Node Wash-out Fluid with TI-RADS to Predict Lymph Node Metastasis in Papillary Thyroid Carcinoma

Xueni Lu[1], Ying Dang[2], Blessed Kondowe[3], Hui Zhang[4], Jin Shang[4], Wenjing Wang[5], and Xiang Wang[2]*

1. Nuclear Medicine Department, The First Affiliated Hospital of Xi'an Jiaotong University, Xi'an, Shaanxi, 710061, China
2. Ultrasound Medicine Department, The First Affiliated Hospital of Xi'an Jiaotong University, Xi'an, Shaanxi, 710061, China
3. Radiology Department, Mzuzu Central Hospital, Mzuzu, Malawi
4. Department of Medical Imaging, The First Affiliated Hospital of Xi'an Jiaotong University, Xi'an, Shaanxi, 710061, China
5. Department of Surgery Intensive Care Unit, The First Affiliated Hospital of Xi'an Jiaotong University, Xi'an, Shaanxi, 710061, China

***Corresponding Author:** Xiang Wang; **E-mail:** wangxiang2016@xjtufh.edu.cn

Abstract

Objective

This study was aimed at investigating if the lymph node aspirated wash-out liquid thyroglobulin level and thyroid imaging reporting and data system (TI-RADS) nodule score can be the predictive factor for cervical lymph node metastasis in patients with papillary thyroid carcinoma (PTC).

Methods

The study included 251 patients with surgically confirmed PTC. All the patients underwent preoperative thyroid and cervical ultrasound examination using ACR TI-RADS classification, fine-needle aspiration biopsy (FNAB) for BRAF V600E gene detection, and thyroglobulin (Tg) detection in lymph node aspiration fluid. The results of these examinations and tests were statistically analyzed. A binary logistic regression model was used to determine the predictive impact of Tg levels, gene mutation status, and TI-RADS nodule score on lymph node metastasis.

Results

Among the enrolled patients, 219/251 (87.25%), had BRAF V600E gene mutations and 132/251 (52.59%) had cervical lymph node metastasis. The Tg level in the lymph node aspiration fluid of patients with metastasis was significantly higher than in those without metastasis (324.94 ± 192.52 ng/mL vs 67.93 ± 136.62 ng/mL, P = 0.000), but there was no significant difference in serum Tg levels between the two groups (27.08 ± 71.60 ng/mL vs 20.73 ± 55.21 ng/mL, P = 0.276). The area under the ROC curve (AUC) for lymph node aspiration fluid Tg was 0.858. Thyroglobulin level has a significant positive effect on lymph node metastasis, with a regression coefficient of 0.003 and P = 0.000 < 0.001. BARF V600E mutation status and TI-RADS nodule score do not have a significant effect on lymph node metastasis, with P-values greater than 0.05.

Conclusion

Thyroglobulin levels of lymph node aspiration fluid has a good predictive value for the diagnosis of cervical lymph node metastasis in PTC patients with larger nodules.

Key words: Papillary thyroid carcinoma; Cervical lymph node metastasis; Thyroglobulin; Thyroid Imaging Reporting and Data System; Ultrasound

Introduction

The identification rate of thyroid nodules has significantly increased in recent years due to advances in high-frequency ultrasound equipment and improved awareness among health professionals[1]. Approximately 7%-15% of these thyroid nodules are malignant[2], with papillary thyroid carcinoma (PTC) accounting for about 84%[3]. Generally, PTC has a good prognosis, but about 35% of the patients still develop cervical lymph node metastasis[4]. Fine-needle aspiration cytology (FNAC) is widely accepted by thyroid surgeons for cytopathological diagnosis of thyroid nodules to assess benign and malignant nature. Studies have shown that the diagnostic concordance rate of FNAC for thyroid nodules can reach 90%[5], but the concordance rate for lymph node metastasis is quite low. Clinical ultrasound is the most convenient and commonly used imaging technique to evaluate lymph node metastases[5], although its sensitivity and specificity are low, at about 30% and 86.8%, respectively[6]. Lymph node FNAC has high accuracy but low sensitivity, so improving the diagnostic rate of lymph node metastasis in PTC is of great clinical importance.

In previous studies, researchers have focused more on the predictive value of BRAF V600E gene mutations for lymph node metastasis, the size, location, extra thyroid extension (ETE), and capsular invasion of the nodule for predicting cervical lymph node metastasis[7,8]. However this study aimed at finding out the value of combining Thyroglobulin levels in lymph node wash out fluid with ultrasound features of thyroid and lymph node nodules using ACR TI-RADS to predict lymph node metastasis in patients with confirmed

papillary thyroid carcinoma.

Methodology

Patient Selection

This study retrospectively collected and analyzed 1479 patients who underwent FNAC for thyroid nodules from January 2019 to December 2021 at The First Affiliated Hospital of Xi'an Jiaotong University. All these patients had at least one or more thyroid nodules that were rated category 4 or 5 by the criteria of ACR TI-RADS and were advised to undergo FNAC. This study eventually enrolled 251 patients who underwent surgery and were pathologically diagnosed with PTC. Those without surgical pathology or malignant tumors other than PTC were excluded. All the enrolled patients underwent thyroid nodules and lymph nodes. FNAC, BRAF V600E gene detection and thyroglobulin (Tg) detection in lymph node aspiration wash-out fluid. All patients' thyroid nodule ultrasound examinations were scored and categorized according to the American College of Radiology Thyroid Imaging Reporting and Data System (ACR TI-RADS). The Ethics Committee of Xi'an Jiaotong University's First Affiliated Hospital (XJTU-ZD10) granted ethical permission for this study.

TI-RADS in Thyroid Nodule Processing

This study utilized ACR TI-RADS reporting system in thyroid ultrasound assessment of the patients suspected to have papillary thyroid carcinoma. ACR TI-RADS uses ultrasonography features such as composition, echogenicity, shape, margin and echogenic foci features of thyroid nodules and assign values from 1 to 3 in each feature then add them up to the thyroid nodules to get scores, and the obtained scores are weighted to obtain the category of the nodules. Based on the size and categories of the nodules, nodules smaller than 10 mm in category 5 or 15 mm in category 4 are advised to do follow up visit, and nodules bigger than 10 mm in category 5 or 15 mm in category 4 are advised to do fine needle aspiration biopsy (FNAB) according to the ACR TI-RADS. All thyroid nodules and cervical lymph nodes were scanned using Canon Aplio i900 ultrasound diagnosing system equipped with i18LX5 high-frequency linear probe. The ultrasound examination method for cervical lymph nodes was the same as that for thyroid nodules. When one of the following sonographic features were detected: 1) liquefaction area in the lymph node; 2) calcification or punctate foci appearance in the lymph node; 3) round shape of the lymph node (longitudinal to transverse ratio < 1.5); 4) abnormally rich color doppler blood flow in the lymph node, metastasis was suspected and required FNAB.

Ultrasound-Guided Thyroid Nodule and Lymph Node Fine-Needle Aspiration Biopsy

All patients in category 4 and 5 with nodule size greater than or equal to 10 mm for category 5 and equal to or greater than 15 mm for category 4 were recommended for FNAB. Sample collection followed the standard procedure practiced at The First Affiliated Hospital of Xian Jiaotong University where a 23 G (0.6 mm) fine needle is inserted into the thyroid nodule under ultrasound guidance, repeatedly punctured until a small amount of tissue fluid appears in the junction of the biopsy needle, then the biopsy needle is removed. A 5 mL syringe filled with air is connected to the biopsy needle and the aspired tissue fluid is quickly expelled onto a glass slide, spread evenly with another glass slide, and then fixed in 95% ethanol. The residual tissue fluid inside the needle is washed repeatedly with buffer solution and placed in a centrifuge tube. The same method is used for lymph node fine-needle aspiration biopsy, and after placing the puncture tissue fluid on the glass slide, 5 μL of puncture tissue fluid is taken and placed in 50 μL of buffer solution for thyroglobulin determination, and the remaining tissue fluid on the glass slide is used for pathological smearing.

Lymph Node Aspiration Fluid Tg Detection

The lymph node aspiration fluid Tg detection was performed using a radioimmunoassay method. 200 μL of lymph node aspiration fluid and buffer solution in an Eppendorf (EP) tube were mixed evenly on a mixer, 100 μL of the mixed solution was added to the Tg-coated tube, then 400 μL of the label was added, mixed, and placed in an incubator for 16-20 hours. After washing with the wash solution twice, the coated tube was allowed to dry, and the counting measurement was performed using a gamma counter.

Cytopathological Analysis and BRAF V600E Gene Detection

After fixation with 95% ethanol, the puncture smears were stained with HE, and the sediment in the centrifuge tube was placed on a slide for Papanicolaou staining. A small amount of tissue fluid from the bottom of the centrifuge tube was used for polymerase chain reaction (PCR), and BRAF V600E gene mutation detection was performed with a genetic test kit.

Statistical Analysis

Statistical analysis was performed using SPSS version 20.0. The comparison of diagnostic results of different methods for lymph nodes was conducted using the chi-square test or Mann-Whitney U test. Quantitative data were displayed as mean ± standard deviation (SD). The postoperative pathological results of all patients were used as the gold standard for diagnosis. The diagnostic efficacy between lymph node FNA thyroglobulin (Tg-FNA) and BRAF V600E gene detection results was compared, calculating the sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of both methods. Finally, the receiver operating characteristic (ROC) curve was drawn for both methods to determine the threshold for predicting lymph node metastasis by lymph node FNA thyroglobulin. A P-value of <0.05 was considered statistically significant. Using lymph node wash-out thyroglobulin, BRAF V600E mutation status, and thyroid nodule TI-RADS score as independent variables, and lymph node metastasis as the dependent variable, a binary logistic regression model was established to analyze the predictive impact of globulin levels, gene mutation status, and nodule TI-RADS score for lymph node metastasis.

Results

All 251 enrolled patients underwent FNA of thyroid nodules and cervical lymph nodes and BRAF V600E gene detection, and thyroglobulin were measured in the aspirated lymph nodes wash-out fluid. Serum thyroglobulin levels were also measured in all patients before biopsy procedure. Out of the 251 enrolled patients, 198 were postoperatively pathologically diagnosed with unilateral PTC and 53 with bilateral PTC. In the BRAF V600E gene detection, 219/251 (87.25%) patients (58 males, 161 females) were detected with BRAF V600E mutations, and 32 patients (5 males, 27 females) were

Table 1. Demographic table of lymph node metastasis of PTC patients

		Male(n = 63)	Female(n = 188)	
Total N = 251	mutation	58	161	$X^2 = 1.751$, P = 0.186
	non-mutation	5	27	
LN metastasis N =132	mutation	38	77	$X^2 = 0.999$, P = 0.317
	non-mutation	3	14	

Table 2. Regression results of binary logistic regression model

Variable	B	Standard error	Wald	Degrees of freedom	Significance	Expe (B)	95% CI of Exp (B)	
							Upper limit	Lower limit
Wash-out fluid Tg	0.003	0.001	25.924	1	0.000	1.003	1.002	1.005
BRAF V600E mutation	-0.183	0.397	0.212	1	0.645	0.833	0.382	1.815
TI-RADS score	0.018	0.071	0.061	1	0.805	1.018	0.885	1.170
Constant	-0.577	0.828	0.485	1	0.486	0.562		

Dependent Variable: Lymph Node Metastasis

Table 3. Table for clinical characteristics

	Metastic LN group	Non-Metastic group	P value
N	132	119	0.449
Nodule Size (mm)	11.48 ± 7.69	9.61 ± 6.65	0.001
Tg-LN (ng/mL)	324.94 ± 192.52	67.93 ± 136.62	0.000
Tg-BLD (ng/mL)	27.08 ± 71.60	20.73 ± 55.21	0.230
TI-RADS Score	10.29 ± 1.90	10.18 ± 2.03	0.674

Tg-LN = Thyroglobulin level in the lymph node aspiration fluid; Tg-BLD = Serum Thyroglobulin level; numbers are displayed as mean ± standard error

not detected with mutations. Similarly, 132/251 (52.59%) patients (41 males, 91 females) were postoperatively pathologically confirmed to have cervical lymph node metastasis. Furthermore, 115/219 (52.51%) patients (38 males, 77 females) had lymph node metastasis and among those without detected mutations, 17/32, (53.13%) patients (3 males, 14 females) had lymph node metastasis.

The Tg level in the lymph node aspiration fluid of patients with metastasis was 324.94 ± 192.52 ng/mL (range 0-500 ng/mL), and the serum Tg level was 27.08 ± 71.60 ng/mL. The Tg level in the lymph node aspiration fluid of patients without metastasis was 67.93 ± 136.62 ng/mL (range 0-500

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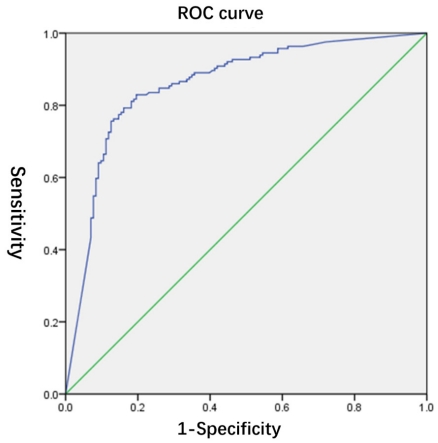

Figure 1. ROC curve of the lymph node aspiration wash-out fluid Tg level

ng/mL), and the serum Tg level was 20.73 ± 55.21 ng/mL (range 0-500 ng/mL). There was a significant statistical difference in lymph node aspiration fluid Tg levels between the two groups, P = 0.000. The area under the ROC curve for lymph node aspiration fluid Tg was 0.858, with a sensitivity of 0.829 and a specificity of 0.804 when the cut-off value was set at 62.99 ng/mL. According to the ACR TI-RADS, the thyroid nodule score for patients with metastasis was 10.29 ± 1.90, and for those without lymph node metastasis, it was 10.18 ± 2.03. The Mann-Whitney U test showed no difference between the two groups (P = 0.674). The size of the thyroid nodules in the group pathologically diagnosed with PTC and lymph node metastasis was approximately 11.48 ± 7.69 mm, and in the group without metastasis was about 9.61 ± 6.65 mm (p = 0.037). The Mann-Whitney U test showed a statistically significant difference between the two groups (P = 0.001). The sensitivity of FNA for the diagnosis of PTC lymph node metastasis was 63.86%, specificity 100%, positive predictive value 100%, negative predictive value 69.65%, and diagnostic efficiency 80.13%. The sensitivity of aspiration wash-out fluid Tg for the diagnosis of PTC lymph node metastasis was 86.59%, specificity 69.93%, positive predictive value 76.76%, negative predictive value 81.97%, and diagnostic efficiency 78.83%.

The regression results showed that Wash-out thyroglobulin level has a significant positive predictive effect on cervical lymph node metastasis, with a regression coefficient of 0.003 and P = 0.000 < 0.001. BRAF V600E mutation and thyroid nodule TI-RADS score did not have a significant effect on lymph node metastasis, with P-values > 0.05.

Discussion

The predictive significance of lymph node aspiration wash-out fluid thyroglobulin level, BRAF V600E gene mutation, ACR TI-RADS score of thyroid nodule ultrasonography features for cervical lymph node metastasis were examined in this retrospective cross-sectional study.

A prospective study conducted in China which included 145 patients with papillary thyroid carcinoma found that BRAF gene mutation has good predictive value for lymph node metastasis[7], but the sample size of the study was relatively small. In a retrospective review analysis, the BRAF V600E gene mutation rate was about 74.6%, which is relatively high[9], and there was a significant difference between the overall metastasis rate. A study conducted on PTC, up to 35% of patients had metastasis[4], and in these patients, the ultrasound metastatic features of the lymph nodes were not obvious. How to specifically and sensitively identify these cervical metastatic lymph nodes before surgery has always been a problem for endocrine and surgical doctors. In recent years, fine-needle aspiration biopsy (FNAB)has become a method for diagnosing the benign and malignant nature of thyroid nodules[10], and combined with the Bethesda system, it can stratify the pathological diagnosis of thyroid nodules[11], with good diagnostic rate and repeatability. However, this method has some shortcomings in the diagnosis of lymph node metastasis.

In this study, we found that lymph node thyroglobulin has better sensitivity and specificity than FNAC, but the diagnostic efficiency between the two is comparable. Similarly, the Tg level in metastatic lymph nodes was higher than in non-metastatic lymph nodes, and there was a significant statistical difference between the two, indicating that this method has good diagnostic value and potential in diagnosing metastatic lymph nodes, consistent with the results of published studies[7,12], but limited by the small sample size of these studies. Despite the good value obtained from the previous results, in this study, we found that the solitary ACR TI-RADS score of thyroid nodules did not show a significant difference in estimating lymph node metastasis. ACR TI-RADS is a commonly used method for radiologists to assess the stratified risk of thyroid nodules in recent years. This method quantifies the ultrasound characteristics of thyroid nodules, including echogenicity, composition, shape, margin, and echogenic foci, and then adds the scores to obtain five risk levels[13]. The higher the nodule score, the greater the risk of malignancy of the nodule. This study attempted to use this method to predict lymph node metastasis with the score of thyroid nodules, but no statistical difference was obtained. One of the main reasons could be that the malignant risk range of TI-RADS category 5 nodules is broad and cannot classify the risk into more detailed levels. Although there was no difference in the TI-RADS scoring system of thyroid nodules between metastatic and non-metastatic lymph nodes, this study still showed that the size of thyroid nodules in metastatic cervical lymph nodes was larger than that of thyroid nodules without cervical lymph node metastasis.

There is still controversy over the overtreatment of low-risk PTC, especially papillary thyroid microcarcinoma. Some researchers believe that active surveillance (AS) is currently the main approach[14], for example, an observational study conducted in Japan found that the progression is very slow[15]. Assessing the lymph node metastasis of PTC in various ways may have a significant impact on whether AS is chosen.

Limitations

This study still has many shortcomings. First, selection bias is inevitable. In addition, our hospital is a referral hospital and the probability of PTC patients having metastasis may be higher than in other hospitals. Similarly, the number of patients in this study is relatively small, and more samples need to be added in the future.

Conclusion

Lymph node FNA for Thyroglobulin level evaluation has good sensitivity and specificity for predicting lymph node metastasis in patients with PTC, combined with ACR TI-RADS classification in patients with larger thyroid nodules.

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Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability statement

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

References

1. Gharib H, Papini E, Paschke R, Duick DS, Valcavi R, Hegedüs L et al. American Association of Clinical Endocrinologists, Associazione Medici Endocrinologi, and European Thyroid Association medical guidelines for clinical practice for the diagnosis and management of thyroid nodules: executive summary of recommendations. J Endocrinol Invest. 2010 May; 33(5 Suppl):51-56. doi: https://doi.org/10.1007/BF03346587.

2. Hegedus L. The thyroid nodule. N Engl J Med. 2004 Oct; 351(17):1764-1771. doi: 10.1056/NEJMcp031436.

3. Lim H, Devesa SS, Sosa JA, Check D, Kitahara CM. Trends in Thyroid Cancer Incidence and Mortality in the United States, 1974-2013. JAMA. 2017 Apr 4;317(13):1338-1348. doi: 10.1001/jama.2017.2719.

4. Schlumberger M J. Papillary and follicular thyroid carcinoma. N Engl J Med. 1998 Jan; 338(5):297-306. doi: 10.1056/NEJM199801293380506.

5. Krišto B, Vidović Krželj I, Krželj A, Perković R. Ultrasound guided fine needle aspiration cytology (FNAC): an assessment of the diagnostic potential in histologically proven thyroid nodules. Med Glas (Zenica). 2022 Aug 1;19(2). doi: 10.17392/1469-22.

6. Hwang HS, Orloff LA. Efficacy of preoperative neck ultrasound in the detection of cervical lymph node metastasis from thyroid cancer. Laryngoscope. 2011 Mar;121(3):487-91. doi: 10.1002/lary.21227.

7. Li J, Liu J, Yu X, Bao X, Qian L. BRAFv600e mutation combined with thyroglobulin and fine-needle aspiration in diagnosis of lymph node metastasis of papillary thyroid carcinoma. Pathol Res Pract. 2018 Nov;214(11):1892-1897. doi: 10.1016/j.prp.2018.09.003.

8. Lu J, Liao J, Chen Y, Li J, Huang X, Zhang H et al. Risk factor analysis and prediction model for papillary thyroid carcinoma with lymph node metastasis. Front Endocrinol (Lausanne). 2023 Oct 30;14:1287593. doi: 10.3389/fendo.2023.1287593.

9. Wei X, Wang X, Xiong J, Li C, Liao Y, Zhu Y, Mao J. Risk and Prognostic Factors for BRAFV600E Mutations in Papillary Thyroid Carcinoma. Biomed Res Int. 2022 May 18;2022:9959649. doi: 10.1155/2022/9959649.

10. Baloch ZW, Cibas ES, Clark DP, Layfield LJ, Ljung BM, Pitman MB, Abati A. The National Cancer Institute Thyroid fine needle aspiration state of the science conference: a summation. Cytojournal. 2008 Apr 7;5:6. doi: 10.1186/1742-6413-5-6.

11. Cibas ES, Ali SZ. The 2017 Bethesda System for Reporting Thyroid Cytopathology. Thyroid. 2017 Nov;27(11):1341-1346. doi: 10.1089/thy.2017.0500.

12. Zhang Z, Zhang X, Yin Y, Zhao S, Wang K, Shang M et al. Integrating BRAFV600E mutation, ultrasonic and clinicopathologic characteristics for predicting the risk of cervical central lymph node metastasis in papillary thyroid carcinoma. BMC Cancer. 2022 Apr 27;22(1):461. doi: 10.1186/s12885-022-09550-z.

13. Tessler FN, Middleton WD, Grant EG, Hoang JK, Berland LL, Teefey SA et al. ACR Thyroid Imaging, Reporting and Data System (TI-RADS): White Paper of the ACR TI-RADS Committee. J Am Coll Radiol. 2017 May;14(5):587-595. doi: 10.1016/j.jacr.2017.01.046.

14. Sugitani I, Ito Y, Takeuchi D, Nakayama H, Masaki C, Shindo H et al. Indications and Strategy for Active Surveillance of Adult Low-Risk Papillary Thyroid Microcarcinoma: Consensus Statements from the Japan Association of Endocrine Surgery Task Force on Management for Papillary Thyroid Microcarcinoma. Thyroid. 2021 Feb;31(2):183-192. doi: 10.1089/thy.2020.0330.

15. Ito Y, Uruno T, Nakano K, Takamura Y, Miya A, Kobayashi K et al. An observation trial without surgical treatment in patients with papillary microcarcinoma of the thyroid. Thyroid. 2003 Apr;13(4):381-7. doi: 10.1089/105072503321669875.